INCORPORATION OF AMINO ACIDS INTO FRACTIONS OF INSOLUBLE COLLAGEN

EVALUATION OF METABOLIC HOMOGENEITY OF COLLAGEN FIBRES

by

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A b s t r a c t: Chick embryos were injected with ^{14}C-amino acids and the embryos harvested after 4 hrs. The insoluble collagen was isolated, gelatinized and fractionated into four fractions in Amberlite CG-50 columns.

The specific activities of the fractions decreased according to the order of the eluates. However, the analysis on the content of hydroxyproline revealed that the latter fractions contain non-collagenous protein impurities, which explain, partly at least, the smaller radioactivities in the latter fractions.

The possibility of metabolic heterogeneity of insoluble biological structures is discussed.

In an earlier work (1), gelatins were divided into an arbitrary number of fractions by eluting Amberlite CG-50 columns with buffers of increasing pH and salt strength (Fig. 2). Those fractions which emerged first, were more acid and of lower molecular weight, *i. e.* more degraded, than the latter. It was expected that the degradation of collagen, in the terms of molecular weight and deamidation, would be a gradual time-dependent process, and therefore the collagen molecules originating from the surfaces of the insoluble fibres would be the most affected and thus emerge first in the Amberlite CG-50 column fractionation.

This hypothesis received support from preliminary findings in connection with other work: when insoluble collagen from chick embryos, injected in advance with either ^{14}C-proline, ^{14}C-leucine or ^{14}C-glutamic acid, was isolated, gelatinized, fractionated and finally analyzed on the specific radioactivity, the patterns shown in Fig. 1 could be collected. It was found, however, that the fractions were contaminated with non-collagenous protein and a repetition of the work seemed justified.

MATERIAL AND METHODS

The chick embryos (hatched for 14 days) were injected with 0.2—0.5 μc of ^{14}C-proline (from the Radiochemical Centre, Amersham, Bucks.) into the yolk sac or on the chorion-allantoic membrane. The incubation at +37°C was continued for 4 hours and the embryos harvested and pooled in groups of five to ten. The embryos were homogenized into about 5-fold volume of 1 *M* sodium chloride solution with a Bühler homogenizer (nominal speed 50,000 rpm) for 2 min. After centrifugation (35,000 *g* for 60 min) the insoluble material was extracted with 5-fold volume of 0.1 *M* citrate buffer, pH 3.8, three times for 24 hours each. All these manipulations were carried out at +4°C. The insoluble collagen of the residue was gelatinized with 5 ml of water per embryo for 3 hours at +130°C in closed tubes. The supernatants were pooled and evaporated in a boiling water-bath.

These gelatin samples were fractionated in Amberlite CG-50 columns into four fractions (Fig. 2). The protein in the fractions was measured with biuret reaction (2) and then hydrolyzed in 6 *N* hydrochloric acid for 3 hours +130°C. The specific activities of ^{14}C-proline and ^{14}C-hydroxyproline were determined by a liquid scintillation method (applying NE 5503 Scintillation Head Unit, Nuclear En-

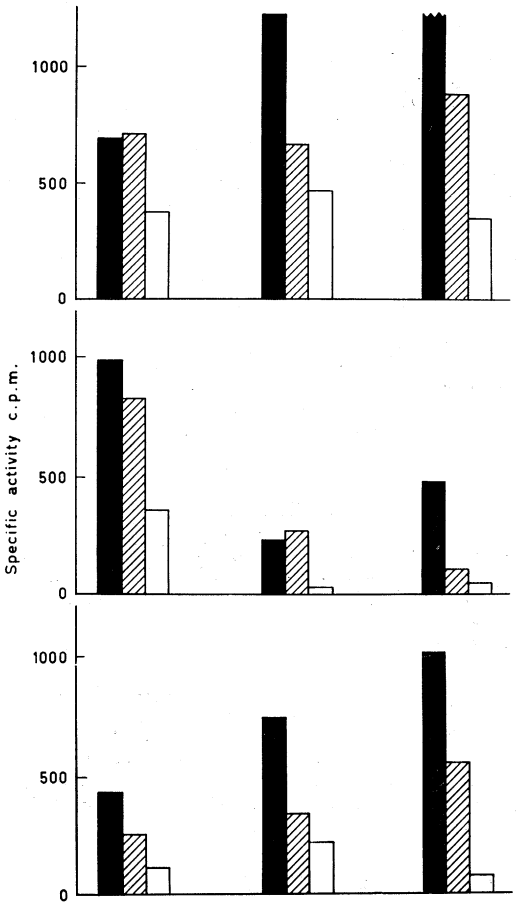

Fig. 1. — Collection of specific radioactivitie (14C per protein in the fraction) in fractions of gelatinized insoluble collagen. In nine separate experiments (nine groups of columns) the chick embryos were treated with 14C-amino acids (proline, leucine or glutamic acid). The insoluble collagen of the embryos was prepared as gelatin, which was separated into three fractions (black, shaded, and open columns in order of emergence) with Amberlite CG-50 column (1) and the radioactivities measured.

terprises Ltd., Edinburgh) according to Peterkofsky and Prockop (3). Both NaOH-eluted fractions were investigated separately, but for Fig. 3 the specific activities were averaged.

RESULTS AND DISCUSSION

The preliminary results which prompted this work have been presented in Fig. 1 and the data from the renewed experiments have been collected in Fig. 3, excluding those where the incorporated radioactivity or the amount of the fractions were too small to be reasonably accurate.

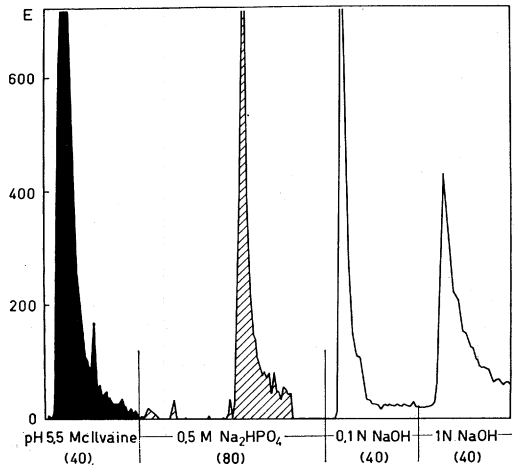

Fig. 2. — Fractionation pattern of gelatinized insoluble collagen of chick embryos using Amberlite CG-50 columns (1). The number of 1-ml fractions is shown in parentheses. The shading of the columns corresponds to that shown in Fig. 1 and in Fig. 3, except that in Fig. 1 and in Fig. 3 the NaOH-eluted fractions (open columns) are combined.

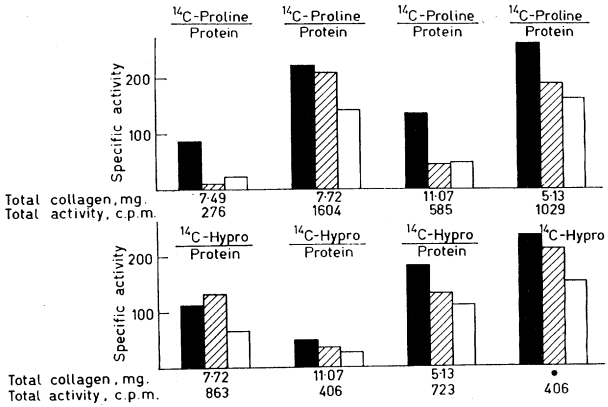

Fig. 3. — Collection of specific radioactivities in fractions of gelatinized insoluble collagen of chick embryos, which had been treated in advance with 14C-proline. The shading of the columns corresponds to the fractions shown in Fig. 1 and in Fig. 2 (NaOH-eluted fractions combined). The specific activities are calculated for 14C-proline and 14C-hydroxyproline (= 14C-Hypro) per mg protein in the fraction, except in one set of fractions (below, extreme right) the specific activities of 14C-hydroxyproline are calculated per amount of hydroxyproline in the fractions.

* Total amount of hydroxyproline 1.98 mg.

The trend in the ^{14}C-activities of the three gelatin fractions was again clear, but the differences were smaller than in the preliminary experiments. Our first impression was that the specific activities seem to decrease with the order of the emergence, and the working hypothesis sounds tenable. The specific activity of hydroxyproline also seems to be in agreement with the working hypothesis. However, our final opinion is somewhat reserved. We believe, on the basis of hydroxyproline analyses, that the variation in the specific activities are partly, but not entirely, due to non-collagen protein impurities in the last fractions, evidenced by their decreasing content of hydroxyproline.

The gelatin in the first fractions from Amberlite CG-50 column was the most pure. The highest activities in the first fractions cannot be due to the ^{14}C-proline of the non-collagenous impurities, because there is an agreement with regard to the activities of ^{14}C-proline and hydroxyproline (Fig. 3).

If the working hypothesis is true, the specific activities of the different fractions will be different, but not necessarily in the order of emergence. The location of the most active concentric layers in the collagen fibres depends on the time interval after the »pulse» of the labelled amino acid.

In any case, caution is to be recommended in the presentation and evaluation of the specific activities of whole, insoluble biological structures, *e. g.*, fibres, bone or teeth, which may not be metabolically homogeneous. The lack of homogeneity can be demonstrated by applying a time-dependent degradation with a suitable, subsequent fractionation as exemplified above.

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